CLINICAL STUDY

Evaluation of bupivacaine-induced muscle regeneration in the treatment of ptosis in patients with chronic progressive external ophthalmoplegia and Kearns–Sayre syndrome

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Abstract

Purpose **Ptosis is common in patients with mitochondrial disease. Whilst surgical shortening of the levator muscle can mechanically elevate the lid, this procedure does not restore normal movement and leaves patients at risk of corneal exposure due to concomitant ophthalmoparesis. Recent studies have shown that bupivacaine-induced muscle regeneration is capable of reversing the molecular genetic and biochemical defect in patients with mitochondrial myopathies. This study was undertaken to assess the potential of this approach in restoring levator muscle function in patients with mitochondrial disease and ptosis.**

Methods **The levator muscle of one eye in five patients with molecularly genetically confirmed mitochondrial DNA disease and ptosis was directly injected with 3 ml of bupivacaine hydrochloride (0.75%). Levator function was compared before and 3 months after the injection.**

Results **No objective clinical improvement in levator function was detected following bupivacaine administration.**

Discussion **The lack of functional recovery seen in our patients is most likely to result from a failure of bupivacaine to induce sufficient regeneration necessary to improve levator muscle function. This result indicates that consideration now needs to be given to the use of alternative and more potent myotoxic agents capable of inducing a more widespread regenerative response from the endogenous muscle satellite cells which contain low or undetectable amounts of mutant mitochondrial DNA.**

Key words Bupivacaine, Chronic progressive external ophthalmoplegia, Kearns–Sayre syndrome, Mitochondria, Muscle regeneration

Defects in mitochondrial DNA (mtDNA) are increasingly being recognised as an important cause of human disease.[1] Ophthalmic involvement is common and typically manifests as either optic atrophy, pigmentary retinopathy, or external ophthalmoplegia with ptosis.[2] Ptosis is the hallmark of patients with chronic progressive external ophthalmoplegia (CPEO) and the related Kearns–Sayre syndrome (KSS). CPEO is typically a benign condition characterised by ophthalmoplegia and ptosis and commonly associated with retinopathy and proximal limb weakness. In contrast, KSS is a multisystem mitochondrial disorder defined by progressive ophthalmoplegia, onset before age 20 years, retinal pigmentary degeneration, and at least one of the following findings: cardiac conduction abnormalities, elevated cerebrospinal fluid protein content, or ataxia.[3] In both patient groups the ptosis is typically slowly progressive,[2] ultimately disrupting vision as the upper lid encroaches across the visual axis. At present there is no effective treatment. Whilst surgical shortening of the levator muscle can mechanically elevate the lid, this procedure does not restore normal movement and leaves patients at risk of corneal exposure due to concomitant ophthalmoparesis. Recent studies,[4,5] however, have suggested an alternative procedure for the treatment of mitochondrial myopathies which offers the potential of restoring levator muscle function.

This new approach is based upon the unique genetic characteristics of mitochondrial muscle disorders. In affected patients, the mtDNA defect is heteroplasmic, that is there is a mixture of both wild-type (normal) and mutant mtDNAs within the same cell, a condition referred to as heteroplasy.[6] Phenotypic changes, however, do not become clinically or biochemically apparent until the ratio of mutant to wild-type mtDNA exceeds a specific

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Table 1. *Clinical diagnosis and laboratory investigations*

Patient no.	Diagnosis	mtDNA defect	Muscle histochemistry		Complex activity			
			%RRF	%COX−ve fibres	I	II	III	IV
1	CPEO	4.9 kb deletion	10	10	Low	N	N	Low
2	CPEO	Multiple deletions	10	20	–	–	–	–
3	CPEO	4.9 kb deletion	2	40	N	N	N	Low
4	KSS	10 kb deletion	10	10	N	N	N	Low
5	CPEO	4.9 kb deletion	0	10	–	–	–	–

Individual respiratory chain activities are designated as being either normal (N) or low according to his laboratory's established standard reference range. In two patients (nos. 2 and 5) biochemical studies were not performed.
CPEO, chronic progressive external ophthalmologia; KSS, Keams–Sayers syndrome; ragged-red fibres; COX−ve fibres, cytochrome-oxidase-negative fibres; kb, kilobase.

threshold level. In limb skeletal muscle, this threshold is between 60% and 85% for a variety of defects in mtDNA.[7–11] Whilst there is a high mutation load in mature muscle fibres, the level of mutant mtDNA is low or even undetectable in myoblasts derived from satellite cells within the same muscle.[4,12–14] Normally quiescent, satellite cells proliferate to form new myofibres in response to muscle fibre degeneration.[15] Local anaesthetics of the aminoacyl group, which includes bupivacaine hydrochloride, are myotoxic to both skeletal and extraocular muscles[16,17] but leave the satellite cell population intact, facilitating rapid regeneration within several weeks.[18,19] By inducing muscle degeneration in this way, Clark *et al.*[5] were able to demonstrate reversal of the biochemical effect of a pathogenic mtDNA mutation in human quadriceps skeletal muscle.

Iatrogenically induced muscle regeneration using bupivacaine hydrochloride appears to offer the potential for restoring muscle function in patients with mitochondrial disease. Target muscles must out of necessity be readily accessible to direct injection and moreover be sufficiently small that regeneration can be safely induced throughout a majority of the constituent myofibres. The levator muscle of the upper lid fulfils these criteria. The finding of low levels of mutant mtDNA in the satellite cells of the levator muscle of a patient with a mtDNA deletion and ptosis[20] further supports the use of this muscle as a clinical model. A limited clinical study was therefore undertaken to investigate the potential role of bupivacaine-induced muscle regeneration in restoring levator function in patients with mitochondrial disease and ptosis.

Materials and methods

Approval for this study was granted by the local research ethics committee. Specific informed consent was obtained from each individual prior to bupivacaine injection.

Patients

The clinical diagnosis of mtDNA disease was confirmed in each patient on the basis of molecular genetic, histochemical and, in three cases, additional biochemical investigations.[1] The clinical features and laboratory findings are summarised in Table 1. Muscle biopsy specimens were examined histochemically with special reference to the incidence of 'ragged-red' fibres (fibres with abnormal subsarcolemmal aggregates of mitochondria) and the incidence of respiration-deficient (cytochrome-oxidase-negative) fibres.[21] Individual respiratory chain complexes I, II, III and IV were measured as previously described.[22] Ages ranged from 30 years to 61 years at the time of injection.

Clinical evaluation of levator function

Lid position and levator function were measured photographically. Levator function was defined as the excursion of the upper eyelid between extremes of upgaze and downgaze. Lid position and palpebral aperture, with and without pressure over the frontalis muscle, were determined in the primary position of gaze from photographs taken with the patient fixing on a distant object at eye level. Photographs were then taken on extremes of upgaze and downgaze to determine levator function, again with pressure applied over the frontalis to negate the action of this muscle. A millimetre scale was taped onto the forehead so that accurate measurements could be made on projected images before and after treatment. In all cases, the observer making the measurements from clinical photographs was masked as to whether the picture was taken before or after injection and to which eye had been treated.

Injection of bupivacaine into the levator/superior rectus complex

All injections were performed in the Ophthalmic Unit Operating Suite, Royal Victoria Infirmary, Newcastle upon Tyne, with full resuscitation facilities available. An indwelling intravenous cannula was inserted prior to injection and pulse and oxygen saturation monitored throughout the procedure with a pulse oximeter.

One eye of each patient was chosen at random. Three millilitres of bupivacaine hydrochloride (0.75%), containing 50 IU hyaluronidase, were injected into the levator/superior rectus muscle complex under electromyographic control. Single injections were performed at the site where the maximal increase in the audible signal was obtained on upgaze. After the induction of complete ptosis was confirmed, the eye was padded and the patient observed on the ophthalmic

Table 2. *Lid position and levator function before and 3 months after bupivacaine injection*

		Pre-injection				Post-injection			
		PA		LF		PA		LF	
Patient no.	Injected eye	Right	Left	Right	Left	Right	Left	Right	Left
1	Left	8	**6**	6	**6**	6	**6**	6	**6**
2	Left	7	**6**	10	**10**	6	**5**	8	**10**
3	Right	**6**	5	**7**	7	**6**	5	**7**	7
4	Right	**3**	6	**2**	5	**3**	5	**2**	5
5	Right	**9**	9	**14**	14	**9**	8	**13**	10

Measurements of both palpebral aperture and levator function are given in millimetres. Numbers in **bold** refer to the injected eye. PA, palpebral aperture; LF, levator function.

ward for several hours. Each individual was given a contact telephone number prior to discharge in the event of any problems occurring prior to their follow-up appointments.

Follow-up and assessment

All patients were seen in the ophthalmic outpatient clinic 3 weeks and 3 months after injection. On each occasion lid position and levator function were measured as described above.

Results

No patient experienced any adverse reaction as a result of bupivacaine injection. In all cases some fullness of the upper lid was noted, suggesting that the bupivacaine had tracked forward in the facial sheath of the levator/superior rectus muscle complex and bathed most of these muscles. In no patient did the induced complete ptosis persist for more than 24 h.

Lid position and levator function for each patient, both before and 3 months after injection, are shown in Table 2. There was no significant change in either of these parameters in any individual. Patients 3 and 4 reported some subjective improvement in lid function. This, however, could not be measured clinically.

Discussion

Previous studies have demonstrated that bupivacaine-induced muscle regeneration is capable of reversing the mtDNA defect in human skeletal muscle by stimulating the proliferation of satellite cells which have low levels of mutation.[5] The results of this study were disappointing, with failure to demonstrate any increase in levator function following bupivacaine administration. Although two patients reported a subjective improvement in lid function, this was not confirmed clinically. It is likely that this represented a placebo effect.

The temporal response of extraocular muscles to bupivacaine injection is similar to that seen in skeletal muscle, with maturation of regenerated muscle fibres observed by day 30.[16,17,19,23] Post-injection biopsy in a patient with mtDNA disease showed regenerating cytochrome *c* oxidase (COX)-positive quadriceps muscle fibres after 3 weeks.[5] Failure of improvement in levator function in this study is therefore not the result of insufficient follow-up for muscle fibre regeneration to have occurred.

Given that none of our patients had any significant increase in ptosis beyond the first 24 h, widespread degeneration of the levator muscle could not have occurred following bupivacaine injection. The most likely explanation, as shown in previous reports of bupivacaine-induced necrosis in quadriceps in man,[5] is that degeneration was patchy despite extensive infiltration. Whilst there is remarkable degeneration/regeneration of some fascicles, these are widely spaced within the muscle. Overall, less than 10% of total skeletal muscle fibres undergo regeneration (K.M. Clark, personal communication). Necrosis may be even more limited in extraocular muscles, with evidence to suggest that they are more refractory than skeletal muscle to the myotoxic side-effects of bupivacaine.[24] The cause of this differential response is unclear, but may be related to the unique structural and functional properties of extraocular muscles.[25] Taken together, this indicates that the lack of clinical improvement seen in our patients is most likely due to a failure of bupivacaine to induce sufficient regeneration necessary to improve levator muscle function.

Muscle necrosis and regeneration nevertheless remains a potential therapy for patients with mitochondrial myopathies, and may be particularly valuable for the treatment of ptosis in patients with CPEO and KSS. The results of this present study suggest that consideration now needs to be given to the use of alternative and more potent myotoxic agents capable of reliably inducing regeneration throughout the whole of the levator muscle.

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